Abstract

Transgenic chloroplast technology could provide a viable solution to the production of Insulin-like Growth Factor I (IGF-I), Human Serum Albumin (HSA), or interferons (IFN) because of hyper-expression capabilities, ability to fold and process eukaryotic proteins with disulfide bridges (thereby eliminating the need for expensive post-purification processing). Tobacco is an ideal choice because of its large biomass, ease of scale-up (million seeds per plant), genetic manipulation and impending need to explore alternate uses for this hazardous crop. Therefore, all three human proteins will be expressed as follows: a) Develop recombinant DNA vectors for enhanced expression via tobacco chloroplast genomes b) generate transgenic plants c) characterize transgenic expression of proteins or fusion proteins using molecular and biochemical methods d) large scale purification of therapeutic proteins from transgenic tobacco and comparison of current purification / processing methods in *E.coli* or yeast e) Characterization and comparison of therapeutic proteins (yield, purity, functionality) produced in yeast or *E.coli* with transgenic tobacco f) animal testing and pre-clinical trials for effectiveness of the therapeutic proteins.

Mass production of affordable vaccines can be achieved by genetically engineering plants to produce recombinant proteins that are candidate vaccine antigens. The B subunits of Enteroxigenic *E. coli* (LTB) and cholera toxin of *Vibrio cholerae* (CTB) are examples of such antigens. When the native LTB gene was expressed via the tobacco nuclear genome, LTB accumulated at levels less than 0.01% of the total soluble leaf protein. Production of effective levels of LTB in plants, required extensive codon modification. Amplification of an unmodified CTB coding sequence in chloroplasts, up to 10,000 copies per cell, resulted in the accumulation of up to 4.1% of total soluble tobacco leaf protein as oligomers (about 410 fold higher expression levels than that of the unmodified LTB gene). PCR and Southern blot analyses confirmed stable integration of the CTB gene into the chloroplast genome. Western blot analysis showed that chloroplast synthesized CTB assembled into oligomers and was antigenically identical to purified native CTB. Also, GM_1-ganglioside binding assays confirmed that chloroplast synthesized CTB binds to the intestinal membrane receptor of cholera toxin, indicating correct folding and disulfide bond formation within the chloroplast. In contrast to stunted nuclear transgenic plants, chloroplast transgenic plants were morphologically indistinguishable from untransformed plants, when CTB was constitutively expressed. The introduced gene was stably inherited in the subsequent generation as confirmed by PCR and Southern blot analyses. Incrased production of an efficient transmucosal carrier molecule and delivery system, like CTB, in transgenic chloroplasts makes plant based oral vaccines and fusion proteins with CTB needing oral administration a much more practical approach.

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